OVARIAN FUNCTION IN HYPOPHYSECTOMIZED RATS

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ELEVEN FIGURES

It is well known that after hypophysectomy in the rat estrous cycles cease and the ovary undergoes marked atrophy. Within 2 to 4 days the large ovarian follicles show signs of degeneration and no new ones develop beyond the early antrum stage (Smith, '30), while within 12 days the theca cells become transformed into plasma or wheel cells (Selye, '33). On the other hand, the corpora lutea regress very slowly (Smith, '30). In this the rat differs markedly from the mouse in which atrophy of the corpora lutea is particularly rapid (Selye, Collip and Thomson, '33). This slow rate of involution in the rat is not affected by the administration of progesterone or follicle stimulating hormone, but treatment with luteinizing preparations of the anterior pituitary cause their rapid disappearance (Bunde and Greep, '36).

In view of these observations Nelson and Merckel ('37) were surprised to find that "dehydroandrosterone" caused almost continuous vaginal cornification for 3 weeks in a not-spayed hypophysectomized rat just as it did in the normal, while in the spayed animal at the same dosage level, the vaginal smear was continuously diestrous. The ovary appeared to be able to transform this substance into a more folliculoid type of compound without the intermediary of the pituitary. Parkes and Zuckerman ('38) observed a similar response to testosterone after hypophysectomy and this was confirmed by Noble ('39) with testosterone propionate. The latter author found that cornification is not elicited in spayed or immature hypophysectomized rats but occurs if precocious puberty is induced with gonadotropic extracts before hypophysectomy. Thus the presence of a corpus luteum appears to be essential although it need not be recently formed since vaginal cornification was observed even if injections were commenced only 10 days after hypophysectomy. In the rat testosterone induces only transitory vaginal estrus (Selye and Friedman, '40), but the stimulation of uterine growth is much

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greater in the intact than in the spayed animal (Korenchevsky and Hall, '37). This suggests that the normal ovary may also transform certain steroids into more folliculoid substances or sensitize the accessory sex organs to these compounds.

Up to the present this particular type of "gonadotropic" effect has only been observed with testoid compounds. Preliminary experiments performed in this laboratory indicated, however, that pregnenolone (Δ^5–17(α)–ethylandrostene–3(β)–ol–17[1]–one), which is practically devoid of testoid activity, also exhibits such an indirect folliculoid effect. Hence it was deemed of interest to study representatives of each independent steroid hormone action (Selye, '42) for their comparative folliculoid effectiveness in hypophysectomized rats with and without ovaries.

EXPERIMENTAL

Three experiments were performed. In the first an investigation was made of the effect of testosterone in relation to the time of the cycle at which hypophysectomy was performed, since in a preliminary (unpublished) experiment, in disagreement with Noble ('39), not all of the animals showed continuously estrus vaginal spreads. The vaginal smears of thirty normal adult female rats weighing about 150 gm. were taken bidaily for 3 weeks. At the end of this time four animals with irregular cycles were eliminated and the remaining twenty-six were hypophysectomized. Starting the day of the operation, the rats were injected bidaily, subcutaneously with 1 mg. of testosterone in 0.1 cc. of peanut oil for 3 weeks. Half of the twenty-two animals surviving at this time were then spayed. The extirpated ovaries were fixed in "Suza", weighed and sectioned. Treatment of both groups was continued with the dose increased to 10 mg. daily. Vaginal smears were taken bidaily throughout. At the end of 10 days the animals were sacrificed and the ovaries, uterus, vagina, adrenals, thymus and preputial glands were removed, fixed in "Suza" and weighed according to our previously described technic (Clarke and Selye, '43). The site of the pituitary was carefully examined for any residual hypophyseal tissue and one doubtful case was eliminated. Ovaries, uterus, vagina and mammary glands were sectioned and stained with hematoxylin-eosin.

In agreement with our preliminary experiment, only about two thirds of the animals showed estrous vaginal smears after hypophysectomy. There was no correlation between the phase of the cycle at which the operation was performed and the occurrence of vaginal cornification.

In no case did the keratinization continue for longer than 5 days and usually it did not last more than 2. After the initial estrous response the smears of all the animals contained large numbers of mucous cells and leukocytes, while a few of them showed irregular short periods of cornification of 24 hours or less. After ovariectomy and the increase in the dose of testosterone, the smears became more and more leukocytic, while in the not-spayed animals there occurred a second short period of transitory estrus followed again by the sporadic appearance of cornified spreads.

At autopsy the ovaries were atrophic and differed neither histologically nor in weight from those of untreated hypophysectomized rats. The adrenal and thymus glands were markedly and equally atrophic in both groups. The average weight of the adrenals was 14 mg. (range 9–20 mg.), while that of the thymus was 23 mg. (range 12–50 mg.). The uteri of the not-spayed animals weighed only slightly more than those of the spayed (1027 mg. as compared with 953 mg.). There was no significant difference between the weights of either the vaginae or the preputial glands of these two groups.

On the other hand, histologically the vaginal epithelium of the spayed group was less mucified than that of the not-spayed. In three animals of the latter group — but in none of the former — this epithelium was found to consist of cornified or stratified squamous cells. That testosterone is more active as a folliculoid in the not-spayed animals was also shown by the fact that the height of the uterine epithelium was 118 μ ± 13 [2] in the spayed, as compared to 180 μ ± 22 in the not-spayed. These means are significantly different from each other.

The mammary glands in both groups showed marked signs of stimulation in disagreement with the observations of Noble ('39) and the generally accepted view that the pituitary mediates the mammotropic effect of steroids. Figure 1 represents the typical atrophic mammary gland of a hypophysectomized female rat, while figure 2 shows the marked stimulation of the breast tissue which was seen in all testosterone treated animals. The ducts are enlarged, the acini appear to have grown somewhat and both are distended with secretion. There is an increase in the fibro-muscular tissue surrounding the ducts. The amount of this tissue enveloping blood vessels, nerves and even lymph channels was also greatly augmented. The degree of stimulation seen

[2] Standard error $= \sqrt{\frac{\Sigma d^2}{n-1}} \div \sqrt{n}$ where d is the difference between the observed value and the mean and n the number of observed values. A difference between two means is considered significant when it is more than twice the square root of the sum of the squares of the standard errors of the means.

here exceeds that recently reported by Leonard ('43) with testosterone or estradiol and resembles much more closely the cystic-glandular development elicited by Astwood, Geschickter and Rausch ('37) and by Herold and Effkemann ('36) in normal male rats or in castrates of both sexes with estrone or testosterone. The extent of the stimulation is by no means comparable to that observed when the pituitary is intact, nevertheless, some development and secretion does occur in the absence of this gland providing that sufficiently large doses are administered. The failure to obtain detectable mammary stimulation with testosterone (McEuen, Selye and Collip, '37) in hypophysectomized rats, was probably due to the fact that the dosages were not sufficiently high to elicit this effect in the absence of the sensitizing hypophyseal stimuli.

Since hypophysectomy appeared to unmask the folliculoid activity of testosterone, it appeared of interest to investigate whether other natural or synthetic steroids would behave in a similar manner. Twenty to twenty-four female rats weighing 150–165 gm. were divided into two groups. The first group was spayed and 4 days later both groups were hypophysectomized. Subcutaneous injections of steroids suspended or dissolved in peanut oil were commenced on the day of hypophysectomy and continued bidaily for 10 days. Vaginal smears were taken daily and the animals were sacrificed 16–18 hours after the last injection. The thymus, ovaries, uterus, vagina and mammary glands were removed and fixed for 24 hours in "Suza", then transferred to 10% formalin. The first four organs were carefully dissected and weighed and the last four sectioned and stained. Thus we examined the effects

Fig. 1 Mammary gland of female rat 10 days after hypophysectomy.

Fig. 2 Mammary gland of female rat 31 days after hypophysectomy, treated with testosterone 2 mg. daily for 21 days and 10 mg. daily for 10 days. Cyst-like dilatation and fibrosis of duct walls.

Fig. 3 Ovary of rat 10 days after hypophysectomy.

Fig. 4 Ovary of rat 10 days after hypophysectomy, injected with Δ^5-pregnenolone 2 mg. daily for 10 days. No change in structure.

Fig. 5 Uterine epithelium of rat hypophysectomized and spayed at the same time and treated with 2 mg. androstenedione daily for 10 days. Epithelium atrophic.

Fig. 6 Vaginal epithelium of same animal as shown in figure 5. Epithelium atrophic.

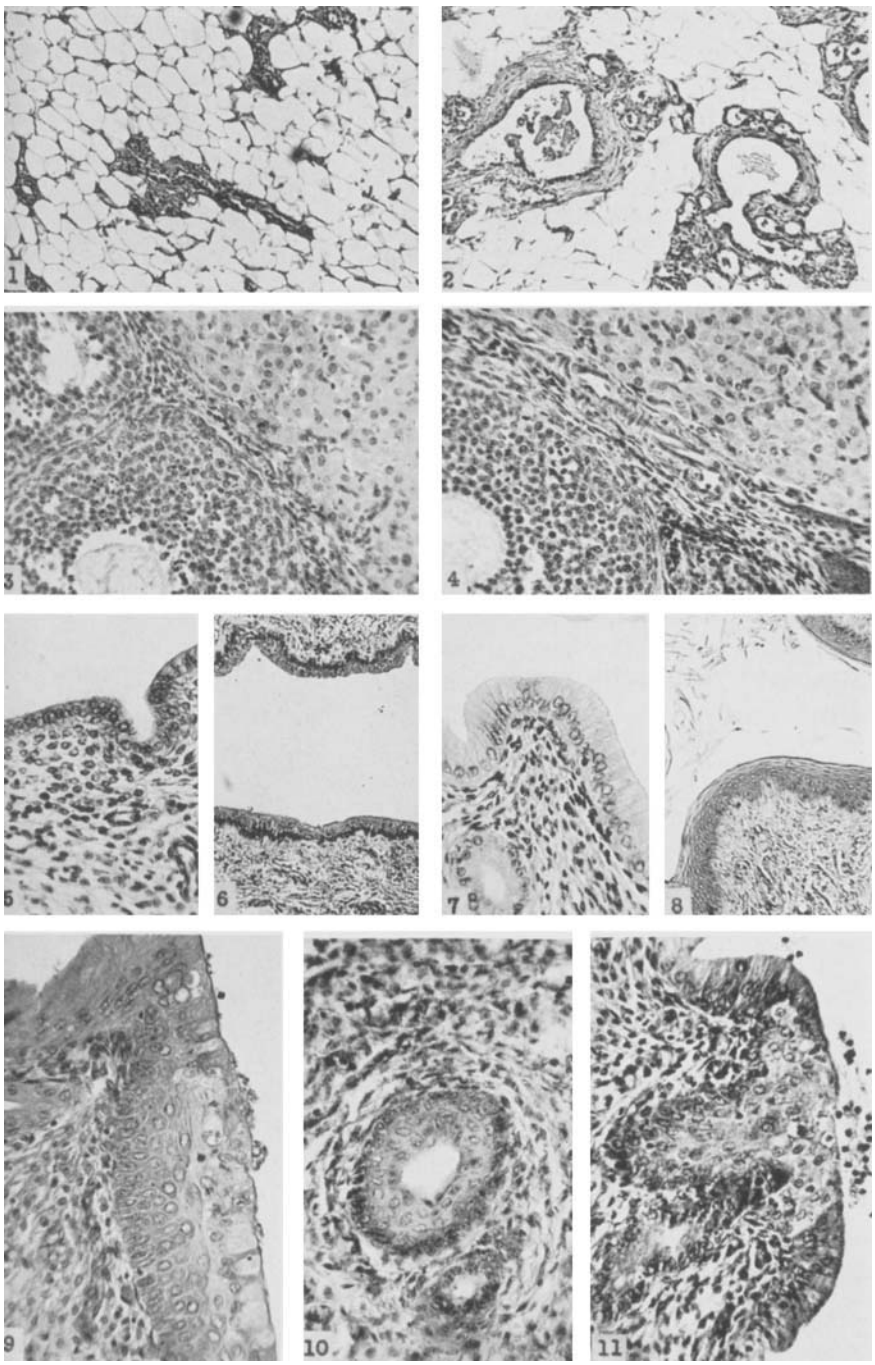
Fig. 7 Uterine epithelium of rat hypophysectomized and treated with 2 mg. androstenedione daily for 10 days. Epithelium high columnar.

Fig. 8 Vaginal epithelium of same animal as shown in figure 7. Complete cornification.

Fig. 9 Stratified squamous cell metaplasia of uterine epithelium of hypophysectomized rat treated with 2 mg. testosterone daily for 21 days and 10 mg. daily for an additional 10 days.

Fig. 10 Metaplasia of the epithelium of a deep uterine gland; note the normal adjacent gland; rat hypophysectomized and treated with Δ^5-pregnenolone for 10 days.

Fig. 11 Glandular cell metaplasia of uterine epithelium of hypophysectomized rat treated with 2 mg. testosterone daily for 10 days.



Figures 1 to 11

of estradiol, progesterone, desoxycorticosterone acetate, pregnenolone, androstenedione, and testosterone at the various daily dose levels indicated in table 1.

It will be noted that ovariectomy was performed 4 days before hypophysectomy in this experiment so that the sensitivity of the tissues of the spayed animals might have been slightly impaired. To investigate whether this would account for the marked differences seen between the spayed and the not-spayed groups, several of the steroids were tested again in an additional experiment in which both groups were hypophysectomized and one ovariectomized simultaneously. In all other respects the procedures in the two series were identical although the doses administered varied in most cases as shown in table 1. The first column of this table indicates the number of the experiment and the second that of the group. In all cases rats of group 1 were spayed, while those of group 2 were not. The steroids used are described by their popular names (in block letters) and by their systematic chemical names according to the recently proposed uniform system of nomenclature (Selye, '43). The melting points of the samples employed are given to facilitate the identification of the compounds and to give a rough estimate of their degree of purity. The dose is expressed as the total amount given daily. The number of animals indicated in each group is the number which lived through the experiment and whose pituitary was known to be completely removed by careful examination at autopsy. The average body weights at this time are also given. The rest of the table is concerned with the various hormonal effects observed. Each figure represents the average within the group and wherever possible standard errors are indicated (brackets). Vaginal smears, although taken daily, are only listed for the first and last days. Cornified spreads are designated as +, while those consisting almost entirely of leukocytes are designated as −. The intermediate state in which the smear contains a mixture of mucous, stratified squamous cells and leukocytes is indicated by ±. The histological structure of the vaginal epithelium at autopsy was recorded by the following abbreviations. A = anestrus, S = slight stratification, S_2 = moderate stratification, S_3 = marked stratification. "C" is used to indicate cornification and "M", mucification, the degrees of these transformations being recorded in the same manner as in the case of stratification. The extent of mammary gland stimulation is expressed in a scale of 0–2 in the next column. 0 indicates no stimulation and an appearance of the mammary gland similar to that pictured in figure 1. 2 represents the same type of cystic development as described above and as shown in figure 2, while 1 indicates an intermediate degree of stimulation of

TABLE 1

*The effect of steroids in spayed and not-spayed hypophysectomized rats.**

EXPERIMENT NO.	GROUP NO.	STEROID	DAILY DOSE	NO. OF ANIMALS	FINAL BODY WEIGHT (G.)	VAGINAL Smear Initial	VAGINAL Smear Final	VAGINAL Histology	MAMMARY GL. STIMULATION	HISTOLOGY OF UTERINE EPITHELIUM (μ)	WEIGHT OF UTERUS (MG.)	WEIGHT OF OVARIES (MG.)
1	1	None peanut oil	0.2 cc	7	142 (2.0)	±	—	A	0	60 (2.6)	**107** (2.5)	
1	2		0.2 cc.	9	152 (2.0)	±	—	A	0	55 (3.2)	140 (7.3)	26 (1.9)
1	1		1 γ	5	128 (3.1)	+	+	S_2C_2	1	131 (19)	**247** (20)	
1	2	$\Delta^{1,3,5}$–estratriene–3, 17(α)–diol α–ESTRADIOL M.P. 176–177°	1 γ	6	136 (4.1)	+	+	S_2C_2	1	175 (14)	337 (15)	28 (2.4)
2	1		0.5 γ	3	136 (3.2)	±	±	S_1C_1	**	279 (18)	425 (50)	
2	2		0.5 γ	6	140 (4.1)	+	±	S_2C_2	**	235 (18)	645 (125)	26 (4.0)
1	1		2 mg.	8	143 (3.5)	—	—	A	0	91 (9.5)	**171** (12)	
1	2	17(α)–[1–ketoethyl–Δ^4–androstene–3–one PROGESTERONE M.P. 128°	2 mg.	8	145 (3.6)	±	—	S_1M_1	0	99 (6.8)	288 (10)	24 (1.5)
2	1		1 mg.	6	132 (13)	±	—	S_1M_1	0	118 (5.0)	150 (5.0)	
2	2		1 mg.	4	141 (12)	±	—	S_1M_1	0	91 (14)	185 (29)	**
1	1		2 mg.	8	148 (2.9)	—	—	A	0	**81** (6.2)	**95** (5.0)	
1	2	17(α)–[1–keto–2–hydroxyethyl] –Δ^4–androstene–3–one acetate DESOXYCORTICOSTERONE ACETATE M.P. 152°	2 mg.	6	149 (3.9)	±	—	A	0	110 (6.8)	144 (9.1)	27 (1.3)
1	1		10 mg.	9	133 (3.6)	—	—	A	0	116 (6.1)	**141** (8.7)	
1	2		10 mg.	10	142 (3.4)	±	—	A	0	**98** (5.0)	195 (12)	25 (1.0)
1	1	17(α)–[1–ketoethyl–Δ^5 androstene–3(β)–ol Δ^5–PREGNENOLONE M.P. 188°	2 mg.	6	145 (4.1)	—	—	A	0	**80** (4.6)	**103** (9.6)	
1	2		2 mg.	5	146 (3.0)	±	+	S_2C_2	0	125 (12)	234 (33)	25 (2.6)
1	1	Δ^4–androstene–3, 17–dione ANDROSTENEDIONE M.P. 169°	2 mg.	5	144 (4.1)	—	—	M_1	2	**74** (4.1)	**217** (26)	
1	2		2 mg.	9	144 (1.8)	±	+	S_2M_2 or C_2	1	251 (21)	406 (22)	26 (3.1)
2	1		2 mg.	7	148 (2.3)	—	—	S_1M_1	2	**84** (6.5)	**286** (29)	
2	2		2 mg.	4	139 (6.5)	±	+	S_3M_3 or C_2	2	206 (18)	741 (287) 429 (39)	25 (1.9)
1	1	Δ^4–androstene–3–one–17(α)–ol TESTOSTERONE M.P. 154°	2 mg.	6	147 (2.0)	—	—	S_2M_1	2	**81** (4.2)	**	
1	2		2 mg.	5	146 (2.0)	±	±	S_2M_3 or C_1	2	145 (15)	407 (40)	24 (2.0)
2	1		2 mg.	9	156 (5.6)	±	—	S_1M_1	2	91 (6.4)	403 (27)	

*For explanations of abbreviations see text. ** Not observed.

the same type. The height of the uterine epithelium in μ and the weights of the uteri and ovaries in mg. are given for each group in the last three columns.

In no case did treatment with these various steroids influence the weight of the ovaries. Histologically there was no difference in the size of the corpora lutea or primary follicles. The various ovarian cells of the treated animals differed in no way from those of the controls as indicated in figures 3 and 4. In the peanut oil treated control animals the height of the uterine epithelium and the vaginal and mammary gland atrophy were not significantly different in the two groups. The uteri of the spayed animals were, however, significantly lighter than those of the not-spayed. This difference in weight may be due in part to the fact that the former were ovariectomized 4 days before hypophysectomy. Yet this circumstance could not explain the observed difference in the uterine weights of the animals in experiment 2. Thus it does not account for the differences seen between the weights of the uteri of spayed and not-spayed androstenedione treated rats. The difference between the weights of this organ in animals spayed 4 days before and on the day of hypophysectomy is not significant, while in both experiments the not-spayed animals had significantly heavier uteri. (In the second experiment the difference was significant if the largest — apparently abnormal — uterus, weighing 1571 mg., were excluded from the calculation. In the table the first mean includes this observation and the second excludes it.) Representative sections of the uterine and vaginal epithelia are shown in figures 5 to 8. With progesterone, on the other hand, a marked metrotropic effect is seen only in experiment 1 at a 2 mg. dose level. The difference at the 1 mg. dose level tested according to the procedure of experiment 2 is not significant. The probability that in this case the difference is due to variations in tissue sensitivity is supported by the identical vaginal responses in the second experiment and by the non-significant differences between the uterine epithelial heights in both. Similarly with desoxycorticosterone acetate the differences between the uterine weights of the spayed and not-spayed groups is perhaps due to this factor. With testosterone the dose was probably too large and maximum growth occurred in both groups. That this substance was more folliculoid in the not-spayed animals is indicated by the markedly significant differences in vaginal responses and in the heights of the uterine epithelia. Pregnenolone was also more folliculoid in the not-spayed than in the spayed rat as shown by the marked difference in uterine weights, epithelial heights and vaginal smears and histology at autopsy. Finally, estradiol appears

to be more folliculoid in the not-spayed than in the spayed rat according to differences in uterine weight and vaginal development (experiment 2) but not by uterine epithelial height. It would be of interest to investigate this point further since if this substance were more active in the presence of the ovary it would suggest that the true ovarian hormone is not estradiol. The present data is merely suggestive. It is interesting that with this compound the lower dose is much more effective in increasing the weight of the uterus than is the higher dose.

In these experiments four instances of uterine metaplasia were observed. Previously, metaplasia of the uterine epithelium has been seen only with excessive and prolonged administration of active estratrienes. Metaplasia with testosterone was seen first in a not-spayed hypophysectomized rat in the first experiment reported above in which large amounts were given for a considerable period of time (fig. 9). In the short-term experiments one case appeared in one of the not-spayed groups of animals treated with testosterone, androstenedione and pregnenolone but not in any of the rats given estradiol or any of the other compounds. The portion of the uterus taken for section was about 5 mm. above the angle of the two horns. Only about eight to ten consecutive sections were cut hence possibly metaplasia occurred more commonly than these observations would suggest. Since serial sections were examined errors due to tangential sectioning could be avoided. The metaplasia was not extensive but patchy. It occurred in the deep glands as seen in figure 10 or in the epithelium of the lumen. The cell-types were either stratified squamous, as seen in figure 7, or resembled mucified vaginal cells as seen in figure 11. In no case did metaplasia occur in any of the spayed animals. Thus the data would suggest that the ovary is able to transform testosterone, androstenedione and pregnenolone into some folliculoid substance which actively promotes uterine epithelial metaplasia.

SUMMARY AND CONCLUSIONS

1. Testosterone, pregnenolone and androstenedione were found to be more folliculoid in the not-spayed than in the spayed hypophysectomized rat. On the other hand, the folliculoid potency of progesterone, desoxycorticosterone acetate and estradiol was only doubtfully increased.

2. Cystic mammary development was observed in hypophysectomized rats with androstenedione, testosterone and, to a much smaller extent, with estradiol.

3. Four cases of uterine metaplasia were observed in not-spayed, hypophysectomized rats with testosterone, androstenedione and pregnenolone.

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